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(54) Method and apparatus for mapping the condition of a wound

(57) The invention provides a diagnostic sheet for mapping the condition of a wound, wherein the sheet is selectively reactive over at least a part of its area with one or more molecules present in a wound fluid. Preferably, the sheet is comprises antibodies reactive with an antigen present in wound fluid, or peptide substrates selectively reactive with protease enzymes present in wound fluid and indicative of wound healing disorders.

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Description

The present invention relates to reactive sheet materials for mapping the condition of a wound, and methods for carrying out such diagnostic mapping.

It is known that large area wounds can show different wound pathologies and different healing rates at different locations on the wound surface. This applies especially to large-area bums, and to large-area chronic wounds such as diabetic ulcers, decubitis ulcers and venous ulcers. Optimization of wound diagnosis and treatment may be assisted by having a map showing the condition of different regions of the wound.

Certain existing methods of determining the condition of a wound rely on analyzing wound fluid that has been removed from the wound surface by a swab or the like. Clearly, the composition of such wound fluid is averaged over the whole surface of the wound, and is not normally representative of any specific area of the wound.

Accordingly, it is an object of the present invention to provide methods for mapping the condition of a wound, in particular for mapping the presence of biochemical wound markers. It is a further object of the present invention to provide an apparatus specifically adapted for use in such a method.

The present invention provides a diagnostic sheet for mapping the condition of a wound, wherein the sheet is selectively reactive over at least a part of its area with one or more molecules present in a wound fluid.

The wound fluid can be transferred directly or indirectly (e.g. by blotting) from the wound surface onto the diagnostic sheet according to the present invention, whereupon the reaction between the molecules present in the wound fluid and the reactive part of the sheet can be used to provide a map of the concentration of the said molecules as a function of position in the wound.

The diagnostic sheet according to the present invention is especially suitable for use with large surface area wounds, for which purpose the reactive area of the diagnostic sheet is sheet preferably greater than about $10cm^2$, and more preferably greater than about $16cm^2$. Preferably, the reactive area of the sheet is substantially round or square. Preferably, the whole of the diagnostic sheet is uniformly reactive with the said molecules present in the wound fluid.

Preferably, the diagnostic sheet contains one or more immunological binding partners to bind the one or more molecules present in the wound fluid. The immunological binding partners may for example comprise monoclonal or polyclonal antibodies, antibody fragments, or chimeric antibodies. Alternatively, the immunological binding partners may comprise antigens in cases where the presence of predetermined antibodies in the wound fluid is being mapped. Preferably, the immunological binding partners comprise monoclonal antibodies. Preferably, the immunological or other binding partners are immobilised on the sheet, for example by avidin-biotin linking, or dialdehyde derivatization of the sheet material followed by cross-linking to a peptide binding partner.

The sheets of material according to the present invention comprising immunological or other binding partners may be used in a range of immunoassays to map the presence of biologically active molecules in large-area wounds. For example, the sheets having antibodies or antibody fragments bound thereto may be used in sandwich immunoassay-type mapping. Alternatively, the sheet may have analog ligands bound to the antibodies, whereby the molecules present in the wound fluid are mapped by affinity displacement immunoassay. Various other immunoassays will be apparent to persons skilled in the art.

In other preferred embodiments, the diagnostic sheet according to the present invention comprises a chemiluminescent, chromogenic or fluorogenic substrate for an enzyme present in the wound fluid.

The sheet of material according to the present invention preferably comprises a plurality of layers. Preferably, these include an absorbent layer for absorbing the wound fluid. The absorbent layer may be the same as, or distinct from a reactive layer for reacting with the one or more molecules present in the wound fluid to map those molecules over the area of the wound. The reactive layer may be located on either side of the absorbent layer (i.e. facing the wound in use, or facing away from the wound in use), provided that wound fluid can pass into or through the reactive layer. The use of an absorbent layer facilitates uptake of the wound fluid and minimizes lateral flow of the wound fluid that could distort the wound map.

In its simplest aspect, the diagnostic sheet according to the present invention is applied to the surface of the wound, for direct absorption of wound fluid over the area of the sheet. For use in such methods the sheet material is preferably conformable, sterile, non-adherent and wound-friendly. Preferably, the sheet further comprises a porous wound contacting layer that permits the flow of wound fluid into the sheet of material, but prevents contamination of the sheet by wound debris. In certain preferred embodiments the wound contacting layer is microporous, with a pore size preferably in the range of 0.01 to 1μm. The use of a microporous film prevents bacterial or particulate contamination of the sheet of material, and may also be useful for some preliminary molecular weight selection of molecules present in the wound fluid. Suitable microporous films include microporous polyurethane films and microporous polyvinylidine fluoride (PVDF) films available from Pall Corporation.

In accordance with a further aspect, the invention provides a diagnostic sheet as above in a sterile package, such as a gamma-irradiated laminated foil pouch.

Preferably, the absorbent/reactive layers of the sheet of material according to the present invention comprise at least one layer of material selected from the

group consisting of: gelatin, cellulose, nitrocellulose, agar, polyacrylamide, starch, alginate, bacterial or plant gums such as guar gum, xantham gum or locust bean gum, glass fiber, polyethylene, polycarbonate or polypropylene.

The sheets of material according to the present invention can be used to map substantially any detectable molecule that is present in wound fluid. The sheets may also be impregnated with an acid/base indicator to map the pH of the wound. Preferably, the sheet is reactive with one or more molecules that are indicative of a wound healing disorder. Preferably, the sheet is reactive with one or more molecules selected from the group consisting of: protease enzymes, collagen propeptides, collagen telopeptides and collagen crosslinks such as pyridinoline, protease inhibitors, plasmin, lactate dehydrogenase, cathepsins, cytokines, peroxidase enzymes, cortisol free radicals and growth factors. More preferably, the one or more molecules are protease enzymes selected from the group consisting of matrix metalloproteinases, low molecular weight gelatinases and latent or active elastases. Most preferably, the one or more molecules comprise matrix metalloproteinase 2 (MMP2) or matrix metalloproteinase 9 (MMP9). The protease enzymes are preferably detected by reaction with chromogenic or fluorogenic substrates bound to the sheet of material, such as Dnp-Pro-β-cyclohexyl-Ala-Gly-Cys(Mu)-His-Ala-Lys(N-Me-Abz)-NH_2 (available from Bachem Inc. - for MMP9 and 1). Other chromogenic substrates such as Gelatin-Texas Red and Gelatin-Azobrilliant Blue could be used to map these molecules.

In other preferred embodiments, the diagnostic sheet reacts with an alkaline phosphatase (ALP) enzyme present in the wound. It is known from F. Alpaslan, T. Nakajima and Y. Takano J. Oral. Maxillofac. Surg. Vol. 55, pp 56-62 (1997) that extracellular ALP levels are elevated in healing wounds. The diagnostic sheet could, for example, comprise a substrate for ALP such as p-nitrophenyl phosphate (see G. N. Price, Clin.Chim. Acta vol. 94, p.211, (1979). Alternatively or additionally, the diagnostic sheet could comprise an immunological binding partner for ALP.

A visible map can also be produced on the diagnostic sheet by various methods known in the art for immunoassay, such as sandwich immunoassay.

In a second aspect, the present invention provides a kit for mapping the condition of a wound, wherein the kit comprises a diagnostic sheet according to the present invention and an immunological binding partner for the one or more molecules, wherein the immunological binding partner is bound to an indicator molecule. Preferably, this is a kit for sandwich immunoassay. The indicator molecule may be any of the indicator molecules conventionally used in the art, such as horseradish peroxidase.

In a third aspect, the present invention provides the use of a diagnostic sheet according to the present invention for the preparation of a diagnostic wound dressing for application to the surface of a wound to map the condition of the wound.

In a fourth aspect, the present invention provides a method of mapping the condition of a wound comprising the steps of: applying an absorbent sheet to the surface of the wound to blot wound fluid from the wound; followed by applying the absorbent sheet to a diagnostic sheet according to the present invention to transfer a part of the wound fluid to the diagnostic sheet for said diagnostic mapping. Preferably, the diagnostic sheet is remote from the wound when the would fluid is transferred to the diagnostic sheet.

Specific embodiments of the present invention will now be described further, by way of example.

Example 1

An absorbent diagnostic wound mapping sheet in accordance with the present invention is prepared as follows. A sheet of Whatman (RTM) No. 1 filter paper (5 cm by 5 cm) is soaked overnight at room temperature in a 1 mM aqueous solution of the elastase chromogenic substrate MeO-Suc-Ala-Ala-Pro-Val-(p-nitroanilide) supplied by Calbiochem Limited. This substrate releases yellow coloured p-nitroanilide dye when the peptide sequence is cleaved by an elastase enzyme. The filter paper is dried at 40°C following the soaking step.

The resulting filter paper has the elastase chromogenic substrate uniformly distributed thereon, and is suitable for mapping elastase concentrations over the surface of a wound.

Procedure 1

The reactivity of the diagnostic sheet prepared in Example 1 is assessed as follows.

Human mastectomy drain fluid both undiluted and diluted tenfold was dropped onto a sample of the diagnostic wound mapping sheet to test its reactivity towards acute wound fluid.

Human venous ulcer fluid both undiluted and diluted tenfold was likewise dropped onto another sample of the diagnostic wound mapping sheet to determine its reactivity towards chronic wound fluid.

It was found that the acute wound fluid did not develop any colour in the wound mapping sheet. The chronic wound fluid produced an intense yellow colour within 60 seconds, indicative of high elastase activity in the chronic wound fluid.

Procedure 2

The diagnostic wound mapping sheet prepared in Example 1 is used for diagnostic wound mapping in the following way.

A first (transfer) layer of Whatman No. 1 (RTM) filter paper is used as a blotting layer to transfer wound fluid from a wound surface to a wound mapping sheet pre-

pared as described in Example 1.

The first layer of filter paper is covered with layer a of Tegapore (RTM) microporous polymer film (supplied by 3M Corporation) to prevent cellular debris being transferred from the wound surface. The first layer with Tegapore (RTM) covering is then applied to the surface of a venous ulcer for 30 seconds to blot wound fluid from the wound surface. The first layer is then removed from the wound, separated from the Tegapore covering, and blotted onto the diagnostic wound mapping sheet. After 30 minutes for colour development, the first layer is removed to leave the diagnostic sheet with a coloured wound map thereon.

The wound map shows regions of the wound where the wound fluid contains elevated quantities of elastase. Such areas are likely to exhibit slower healing and ulceration, since the high levels of elastase interfere with the delicate balance of tissue formation and breakdown required for wound healing.

The above embodiments have been described by way of example only. Many other embodiments falling within the scope of the accompanying Claims will be apparent to the skilled reader.

Claims

1. A diagnostic sheet for mapping the condition of a wound, wherein said sheet is selectively reactive over at least a part of its area with one or more molecules present in a wound fluid.

2. A diagnostic sheet according to Claim 1, wherein said at least part of its area is greater than 10cm^2.

3. A diagnostic sheet according to Claim 1 or 2, which comprises one or more immunological or other binding partners for the said one or more molecules.

4. A diagnostic sheet according to Claim 3, wherein said binding partners are immobilised on said sheet.

5. A diagnostic sheet according to Claim 3 or 4, wherein the binding partners have an analog ligand bound thereto, whereby said sheet is suitable for affinity displacement immunoassay.

6. A diagnostic sheet according to Claim 3, 4 or 5, wherein said binding partners comprise monoclonal antibodies or monoclonal antibody fragments.

7. A diagnostic sheet according to Claim 1, comprising a chemiluminescent, chromogenic or fluorogenic substrate for an enzyme to be mapped in said wound fluid.

8. A diagnostic sheet according to Claim 7, wherein said enzyme is a protease enzyme, and said substrate comprises a peptide.

9. A diagnostic sheet according to any preceding Claim, wherein said sheet comprises an absorbent layer for absorbing a wound fluid.

10. A diagnostic sheet according to Claim 9, wherein the sheet is suitable for application directly to the surface of a wound.

11. A diagnostic sheet according to Claim 10, wherein the sheet further comprises a porous wound contacting layer.

12. A diagnostic sheet according to Claim 11, wherein the wound contacting layer is microporous.

13. A diagnostic sheet according to any preceding claim, comprising at least one layer of material selected from the group consisting of gelatin, cellulose, nitrocellulose, agar, polyacrylamide, starch, alginate, bacterial or plant gums, glass fiber, polyethylene, polycarbonate or polypropylene.

14. A diagnostic sheet according to any preceding claim, wherein said one or more molecules with which the sheet of material is reactive are selected from the group consisting of protease enzymes, collagen propeptides, collagen telopeptides, collagen crosslinks such as pyridinoline, protease inhibitors, plasmin, lactate dehydrogenase, cathepsins, cytokines, peroxidase enzymes, cortisol free radicals and growth factors.

15. A diagnostic sheet according to Claim 14, wherein said one or more molecules are protease enzymes selected from the group consisting of matrix metallaproteinases, low molecular weight gelatinase and elastase.

16. A diagnostic sheet according to any of Claims 1 to 13, wherein the said one or more molecules comprise an alkaline phosphatase enzyme.

17. A diagnostic sheet according to any preceding claim, wherein the concentration of said one or more molecules is indicative of a wound healing disorder.

18. A kit for mapping the condition of a wound, said kit comprising a diagnostic sheet according to any of Claim 1 to 17, and an immunological binding partner for said one or more molecules, wherein the immunological binding partner is bound to an indicator molecule.

19. Use of a diagnostic sheet according to any of

Claims 1 to 17 for the preparation of a diagnostic wound dressing for application to the surface of a wound to map the condition of the wound.

20. A method of mapping the condition of a wound comprising the steps of:

applying an absorbent sheet to the surface of the wound to blot wound fluid from the wound; followed by
applying the absorbent sheet to a diagnostic sheet according to any of Claim 1 to 17 to transfer at least a part of said wound fluid to said sheet of material.

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EUROPEAN SEARCH REPORT

Application Number EP 98301783.1

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl. 6)
D,Y	GÖKHAN, A et al. "Extracellular Alkaline Phosphatase Activity as a Possible Marker for Wound Healing: A preliminary Report" Journal of Oral and Maxillofacial Surgery, January 1997, Vol. 55, No. 1, pages 56-62, abstract. --	1,7, 13,16, 17,19, 20	G 01 N 33/52 G 01 N 33/53 G 01 N 33/573 G 01 N 33/68 C 12 Q 1/00 C 12 Q 1/37 C 12 Q 1/28 C 12 Q 1/32 C 12 Q 1/42
Y	DE 3715245 A1 (FUJI PHOTO FILM CO., LTD.) 19 November 1987 (19.11.87), the whole document. ----	1,7, 13,16, 17,19, 20	

TECHNICAL FIELDS SEARCHED (Int. Cl. 6)

G 01 N 33/00
C 12 Q

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
VIENNA	20-05-1998	SCHNASS

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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